PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet)	
Applicant's or agent's file reference see form PCT/ISA/220	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/051726	05.08.2004	05.08.2003

International Patent Classification (IPC) or both national classification and IPC
G01N33/569

Applicant
INSTITUTO NAZIONALE PER LE MALATTIE INFETTIVE ...

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

European Patent Office - P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk - Pays Bas
Tel. +31 70 340 - 2040 Tx: 31 651 epo nl
Fax: +31 70 340 - 3016

Authorized Officer

Schalich, J

Telephone No. +31 70 340-8915

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/EP2004/051726

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☒ the entire international application,

☐ claims Nos.

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the whole application or for said claims Nos.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form ☐ has not been furnished

☐ does not comply with the standard

the computer readable form ☐ has not been furnished

☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has:

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☒ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. Invention 1: claims 1-21,23-26, 44-46, 58, 67, 69 (in part) and Invention 26: Claims 1-19, 27-28, 40-41, 58, 61, 64, 67-68 (in part)

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	24-26, 44-46,67,69 (for SEQ. ID. 83)
	No:	Claims	1-21, 23, 27-28, 40-41, 58, 61, 64 and 67-68
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-21,23-28,40-41,44-46,58,61,64,67-69
Industrial applicability (IA)	Yes:	Claims	1-21,23-28,40-41,44-46,58,61,64,67-69
	No:	Claims	

2. Citations and explanations

   see separate sheet

Reference is made to the following documents:

D1: FOSTER B. AND PRUSSMAN C.: "Detection of Intracellular Cytokines by Flow Cytometry" 2002, JOHN WILEY & SONS, INC.: "CURRENT PROTOCOLS IN IMMUNOLOGY" , NEW YORK

D2: AMICOSANTE MASSIMO ET AL: "Computer-based design of an HLA-haplotype and HIV-clade independent cytotoxic T-lymphocyte (CTL) assay for monitoring HIV-specific immunity." MOLECULAR MEDICINE (BALTIMORE), vol. 8, no. 12, December 2002 (2002-12), pages 798-807

D3: MAECKER H T ET AL: "Use of overlapping peptide mixtures as antigens for cytokine flow cytometry" JOURNAL OF IMMUNOLOGICAL METHODS, ELSEVIER SCIENCE PUBLISHERS B.V.,AMSTERDAM, NL, vol. 255, no. 1-2, 1 September 2001 (2001-09-01), pages 27-40

D4: DREXLER I ET AL: "Identification of vaccinia virus epitope-specific HLA-A0201-restricted T cells and comparative analysis of smallpox vaccines" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 100, no. 1, 7 January 2003 (2003-01-07), pages 217-222

D5: TERAJIMA MASANORI ET AL: "Quantitation of CD8+ T cell responses to newly identified HLA-A0201-restricted T cell epitopes conserved among vaccinia and variola (smallpox) viruses" JOURNAL OF EXPERIMENTAL MEDICINE, TOKYO, JP, vol. 197, no. 7, 7 April 2003 (2003-04-07), pages 927-932

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Due to the lack of unity of invention and the failure to pay further fees only **claims 1-26, 44-46, 58, 67, 69** (as far as peptide with SEQ. ID. 83 is concerned) and **claims 1-19, 27-28, 40-41, 58, 61, 64, 67-68** (as far as peptide with SEQ. ID. 1 is concerned) were searched and will be consequently the subject-matter of this opinion.

Re Item IV

Lack of unity of invention

This Authority considers that there are at least 54 inventions covered by the claims indicated as follows:

1-20: Claims 1-21,23-26, 44-46, 58, 67, 69 (in part): Variola and derived peptides

21: Claims 1-21,23-26, 42-43, 46, 58, 67,69 (in part): Bacillus Anthracis and derived peptides

22: Claims 1-21, 23-24, 26, 58, 65, 67,69 (in part): Yersinia pestis and derived peptides

23: Claims 1-21, 23-24, 26, 58, 65, 67,69 (in part): Francisella tularensis and derived peptides

24: Claims 1-21, 23-26, 31-41, 46, 58-59, 67-69 (in part): SARS and derived peptides

25: Claims 1-21,23-26, 58, 67,69 (in part): human non-SARS Coronavirus

26-45: Claims 1-19, 27-28, 40-41, 58, 61, 64, 67-68 (in part): HIV and derived peptides

46: Claims 1-19, 29-30, 40-41, 58, 62, 63, 67-68, 72-73 (in part): CMV and derived peptides

47: Claims 1-19, 26, 47, 58, 60, 67 (in part): enteric infections and derived peptides

48: Claims 1-19, 26, 48-58, 66-67, 70-71 (in part): Tumour antigens and derived peptides

49: Claims 1-19, 26, 58-59 (in part): respiratory infections, except SARS

50: Claims 1-19, 26, 58, 61 (in part): sexually transmitted diseases, except HIV

51: Claims 1-19, 26, 58, 62, 72 (in part): in utero infections, except post transplant infections and CMV

52: Claims 1-19, 26, 58, 63, 73 (in part): post transplant infections except CMV

53: Claims 1-19, 26, 58, 64 (in part): blood-borne diseases, except sexually transmitted diseases and HIV

54: Claims 1-19, 22, 26, 58 (in part): bacterial toxins

The reasons for which the inventions are not so linked as to form a single general inventive concept, as required by Rule 13.1 PCT, are as follows:
The single general concept identified are methods and compositions for T cell immunodiagnosis.

Methods for T cell immuno-diagnosis, based on flow cytometry are however profoundly not novel (see documents D1-D3).
Moreover, methods to design peptides for use in T cell immuno-diagnosis, according to claim 19 are profoundly not novel: see document D2, D4 (p 218, co 2, par 2) and D5.
D2, D4 and D5 moreover describe peptides from HIV and Variola, T cell epitopes, derived from various HIV and Variola antigens. Therefore, each HIV and Variola derived peptide constitutes an invention on its own.
Further splitting-up of the other groups of inventions would have been to be expected according to the HIV and Variola group.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Art. 33(2) PCT

The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of **claims 1-21, 23, 27-28, 40-41, 58, 61, 64 and 67-68** is not new in the sense of Article 33(2) PCT.

1.1. Generic claims
Claims 1-18 concern routine techniques for intracellular cytokine staining, as described in documents D1-D3,
Claim 19 refers to a method of predicting probable T cell epitopes, as exemplified in D2, D4 and D5.
D1 to D3 moreover describe the technical features of the kit according to present **claim 58**, rendering said claim not novel.
Claim 24 refers to a software, which identifies peptide-mixtures for immuno-diagnosis, according to claim 19. Most steps concern well-known computer-algorithms, nevertheless, the combination was not disclosed in the state of the art.

1. 1. Variola and derived peptides

D4 and D5 apply the method of claim 19 to Variola, rendering **claims 20-21** not novel.

Claims 25, 26, 44-46, 67 and 69, as far as the peptide with SEQ.ID. 83 is concerned, are novel.

1.3. HIV and derived peptides

Claims 27-28, 40-41, 61, 64 and 67-68 are not novel, when relating to a peptide with SEQ. ID. 1, since said peptide is known from documents D2 and D3.

2. Article 33(3) PCT

The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of **claims 25, 26, 44-46 and 67-69** does not involve an inventive step in the sense of Article 33(3) PCT.

The peptide with SEQ.ID. 83 represents a T cell epitope, which was identified by a well-known method according to present claim 19. Therefore, the T cell epitopes identified by said method can not be inventive and **claims 25, 26, 44-46** lack an inventive step.
Also the use of said peptide, according to present **claims 67-69** is obvious, since the methods how to use said peptides are well-known.

Since the method described in claim 19 is well known in the art (D2), carrying all the steps out by computer, is considered obvious. Therefore, **claim 24** lacks an inventive step.